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HIGH TOLERANCE TO SNAKE VENOM BY THE VIRGINIA OPOSSUM, *Didelphis virginiana*

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Varying degrees of resistance to snakebite by certain animals have been reported. Calmette devoted a chapter to this phenomenon in his classical work *Les Venins* (1907) with particular emphasis on the mongoose. Earlier publications (Lewin, 1898; Phisalix and Bertrand, 1895a, 1895b, 1899; Phisalix, 1922, 1938) reported experiments on immunity. Immunity by certain animals was also reported by Vellard (1949).

Earlier literature also cites the greater resistance of mongooses to cobra venom, meerkats to Cape cobra venom (Grasset *et al.*, 1946) and cangamas to the venoms of South American species (Vellard, 1949). A degree of immunity has also been attributed to genets and domestic cats, correlating with the higher resistance among carnivorous animals (Fraser, 1896). The alleged immunity of wild and domestic pigs in the U.S.A. can be attributed to their tough skins and thick layers of subcutaneous fat which retards systemic absorption. South African pigs, however, are considerably more resistant to cobra and puff adder venoms than are sheep (Grasset *et al.*, 1946).

Various authors have reported on the natural immunity of certain non-poisonous snakes (Philpot and Smith, 1950; Philpot, 1954; Bonnett and Guttman, 1971; Juratsch and Russell, 1971). Information on this topic has been summarized by Minton (1974) and Minton and Minton (1969).

During the period 1968–1974, a casual field study on the comparative effects of envenomation by the eastern diamondback rattlesnake, *Crotalus adamanteus*, on mammals indigenous to the Everglades region was carried out by S. G. Seashole while serving as a youth counselor in the National Park. A natural bite was observed in the field by a 160 cm eastern diamondback on an adult opossum, *Didelphis virginiana*. The opossum displayed no apparent distress and this suggested a remarkable tolerance by that animal to envenomation. In order to ascertain if an actual envenomation did take place, Mr. Seashole conducted field experiments by manually causing snakes to inflict actual bites on captured opossums. None of the bites caused visible signs of distress to the opossums. Mr. Seashole then proceeded to inject measured aliquots of extracted venom in 1 and 2 cm^3

doses intramuscularly and observed no signs of distress. Mr. Seashole reported his observation to the author who conducted a literature search which was non-productive regarding this observation. It is interesting to note that the opossum is considered immune to snakebite by many rural folk of the southeastern United States. On the basis of Mr. Seashole's observation and subsequent inquiries, this study was made.

Adult opossums were collected in central Maryland by the staff of Biologicals Unlimited, Inc. Live snakes and lyophilized venoms were provided by Biologicals Unlimited. An E & M physiograph recorder was provided by Lt. Col. James A. Vick, Dept. of Neurophysiology, Biomedical Labs, Edgewood Arsenal, MD.

Five adult opossums were anaesthetized initially with 50 mg per kg pentobarbitol sodium. In all cases, due to rapid metabolic clearing of the anaesthetic, the opossums required continuous infusion of pentobarbitol to maintain narcosis. When sufficiently anaesthetized, heart rate was noted to be 166 beats per min and respirations, 12 per min. Natural bites were inflicted by causing hand held snakes to bite the shaven anterior portion of the right thigh. Bites were inflicted using (a) eastern diamondback rattlesnake, *Crotalus adamanteus*, (b) timber rattlesnake, *Crotalus h. horridus*, (c) cottonmouth moccasin, *Agkistrodon p. piscivorus*, (d) Russell's viper, *Vipera russelli*, and (e) common Asiatic cobra, *Naja n. kaouthia*.

None of the five opossums developed observable local reactions other than trauma attributable to fang penetration and none developed observable systemic effect, exhibiting negligible alteration of heart rate and respiration. Each of the five opossums recovered rapidly from the anaesthetic and showed no ill effects. This experiment was repeated on three successive occasions using different opossums and different specimens of the same five species of snakes for each bite, a total of 15 opossums and 15 snakes. All had similar results, an apparently negligible effect. All opossums were released following 5 days of observation.

Following this initial study and with full knowledge that actual snakebite is highly variable with regard to amount of venom injected (other than our statistics of average yields), an adult opossum weighing 4·1 kg was transported to the Department of Neurophysiology, Biomedical Labs., Edgewood Arsenal, Maryland. The opossum was anaesthetized with 50 mg per kg pentobarbitol sodium. Arterial blood pressure was monitored using a polyethylene catheter inserted into the femoral artery and connected via a Statham pressure transducer to an E & M physiograph recorder. Respiratory rate, electrocardiograph (EKG) and heart rate were monitored via needle tipped electrodes placed in both sides of the chest wall and connected to the physiograph. The right anterior thigh of the opossum was shaved and allowed to receive the full bite of a hand held cottonmouth moccasin, *Agkistrodon p. piscivorus*, of approximately 130 cm in length. Immediately following envenomation, there was a drop in arterial blood pressure of only 5 mm Hg from a norm of 140/105 to 135/100 mm Hg, and recovery was observed within 10 min. Heart rate increased from 160 to 180 per min, while respirations were unaffected and remained at 12 per min. Continuous infusion of anaesthetic was necessary to maintain narcosis. After 30 min, all parameters returned to normal limits. The site of envenomation displayed about 1–1·5 cm of erythema surrounding the fang punctures, but no noticeable edema, ecchymosis or necrosis. Due to the difficulty in determining the amount of venom injected, a second opossum of 3·6 kg in weight was anaesthetized and lyophilized moccasin venom, reconstituted with 0·9% physiological saline, was injected via the tail vein in a dose of

15 mg per kg. The dosage corresponds with more than five lethal doses for 15 kg dogs as reported by VICK (1973). Again, there was an immediate drop in arterial pressure by no more than 10 mm Hg. The heart rate increased from 164 to 186 per min. Respirations were unaffected. Arterial blood pressure returned to normal limits (Mean, 120) with no further physiological effect after 30 min. This same animal was given an additional bolus of 100 mg of venom, reconstituted in 10 cm³ 0·9% physiological saline with similar results. After 24 hr, the apparently healthy opossum was sacrificed and a necropsy performed. Gross organ pathology was negative.

The Virginia opossum, *Didelphis virginiana*, demonstrates a remarkable physiological tolerance to both 'natural' snakebite and massive i.v. infusion of venom. This observation has not been reported for the opossum in previous literature. No references could be found of similar observations regarding other marsupials. This polyprotodont marsupial is a primitive but also very successful mammal. The opossums of varying species are the only marsupials surviving in the placental world, the predominant marsupial and monotreme mammals of Australia having probably survived due to their isolation. The opossum has remained unchanged for millions of years and probably reached his peak of evolutionary specialization several millions of years ago. The opossum has been observed to prey on small venomous snakes in South America. It is the author's opinion that among the many specializations that have allowed this archaic animal to compete and survive is a unique and extremely efficient immune-response system. With the lack of present evidence implicating instant antibody synthesis or an unknown serum factor, there may be two basic mechanisms responsible for this phenomenon: (a) the molecular targets for the toxins simply are not there; (b) something in tissue may inactivate the toxins before they reach their targets. As a variation on the latter, perhaps it is lack of an activator rather than presence of an inactivator in the tissues (Minton, S. A., personal communication, 1975).

Only further studies will shed more light on these suggestions. The author and Lt. Col. James A. Vick intend to carry out broader studies on the opossum's resistance to snake venoms by measuring additional parameters under anaesthesia, such as electroencephalogram (EEG) and body temperature. Definitive histological and blood studies will also be made. A more extensive report will be forthcoming when these studies, now under way, are complete.

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